REMARKS

These remarks are responsive to the Office action dated October 4, 2005, and are being filed under 37 C.F.R. § 1.111. Claims 50-59 and 61-66 are pending in the application. In the Office action, the Examiner rejected all of the pending claims as being obvious under 35 U.S.C. § 103(a):

- Claims 50-52, 54, 55, 57, 59, and 62-65 were rejected over U.S. Patent No. 6,287,774 to Nikiforov ("Nikiforov") in view of Zhou, et al. "Detection and Sequencing of Phosphopeptides Affinity Bound to Immobilized Metal Ion Beads by Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry", *J. Am. Soc. Mass Spectrom.*, 2000, 11, pp. 273-282 ("Zhou");

- Claim 56 was rejected over Nikiforov in view of Zhou and further in view of U.S. Patent No. 6,022,708 to de Sauvage et al.;

- Claims 53, 58, and 66 were rejected over Nikiforov in view of Zhou and further in view of U.S. Patent No. 5,424,190 to Fuller; and

- Claim 61 was rejected over Nikiforov in view of Zhou and further in view of U.S. Patent No. 5,776,487 to Maxfield Wilson et al.

Applicants traverse the rejections. Applicants contend that the rejected claims are not obvious. In support of this contention, applicants have presented arguments and data showing that (1) the cited references teach away from the claimed invention, and (2) the claimed invention provides unexpected benefits. These benefits, which include a substantial increase in luminescence intensity upon phosphate-gallium binding, as opposed to a substantial decrease or quenching in luminescence intensity upon phosphate-iron binding, are neither taught nor suggested by the prior art. Moreover, these benefits may provide truly dramatic differences in the timing and sensitivity of associated assays. Accordingly, applicants respectfully request reconsideration of the rejected claims, and prompt issuance of a Notice of Allowability.

I. Rejections under 35 U.S.C. § 103

In the Office action, the Examiner rejected claims 50-59 and 61-66 under 35 U.S.C. § 103(a) as being unpatentable over Nikiforov and Zhou (claims 50-52, 54, 55, 57, 59, and 62-65) or further in view of an additional reference (claims 53, 56, 58, 61, and 66). Applicants traverse the rejections. It would not have been obvious to combine the cited references because (1) the references teach away from the subject matter of the pending claims, and (2) the subject matter of the pending claims provides unexpected benefits that rebut any *prima facie* case of obviousness.

Claim 50 is an independent claim from which all the other pending claims ultimately depend. Claim 50 is directed to a method and recites:

> 50. (Previously Presented) A method of detecting addition or removal of a phosphate group to or from a substrate, comprising:
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> contacting a luminescent peptide with a binding partner that binds specifically to the peptide only if the peptide is phosphorylated, wherein the binding partner includes gallium involved in binding between the binding partner and the peptide, and wherein the peptide is a substrate for an enzyme that catalyzes addition or cleavage of a phosphate group to or from the peptide; and
>
> measuring luminescence polarization from the luminescent peptide, wherein the amount of measured luminescence polarization can be related to the extent of binding between the luminescent peptide and the binding partner.

The Examiner rejected claim 50 as being obvious over Nikiforov in view of Zhou. In particular, the Examiner suggested that Nikiforov teaches all of the elements of claim 50 except a binding partner that includes gallium. In addition, the Examiner stated that Zhou provides "the motivation to use gallium as the metal ion."[1] Applicants assert that it

[1] Office action, dated October 4, 2005, pg. 3, last paragraph.

would not have been obvious to combine Nikiforov with Zhou, to replace iron with gallium, for the reasons set forth below.

A. *Zhou Teaches Away from the Use of Gallium in the Binding Assays of Nikiforov*

The Examiner appears to have relied on a statement in the background section of Zhou to provide the motivation for combining Nikiforov with Zhou, as indicated by the following statement from the Office action:[2]

> Zhou et al. teach that immobilized metal ions, such as Fe^{3+} bind with high specificity to phosphoproteins and peptides, and that Ga^{3+} (i.e., a gallium cation) has been discovered as having better selectivity for the phosphopeptides (page 274, left column, last paragraph).

Applicants believe that the Examiner is misinterpreting and therefore misapplying both the cited passage and the Zhou reference. In particular, the "better selectivity" of gallium mentioned in Zhou does not mean better binding, as would be pertinent to a binding assay, but rather a better combined ability to engage and release for subsequent use in mass spectrometry analysis. Thus, rather than providing motivation, Zhou actually teaches away from the use of gallium in place of iron for the binding assays of Nikiforov.

Zhou specifically relates to detection of phosphopeptides that are affinity bound to beads via immobilized metal ions. In Zhou, the phosphopeptides are detected by matrix-assisted laser desorption/ionization mass spectrometry. *Zhou does not disclose any direct measure of binding alone.* Instead, Zhou relates to detection of phosphopeptides that are (1) first bound to immobilized metal ions and (2) then released (e.g., via exposure to a laser and/or a phosphatase enzyme) for mass spectrometry. Based on this approach, Zhou compares the detected signals from peptides after binding to, and release from, iron versus gallium. In particular, Zhou

[2] Id.

detects singly phosphorylated peptides, with or without extensive washing of the beads, and multiply phosphorylated peptides, with or without phosphatase treatment of the beads.

Zhou concludes that Ga^{+3} has better overall "selectivity" for the mass spectrometry assay being used. However, with regard to binding itself, Zhou suggests that Fe^{+3} binds phosphopeptides with higher affinity than Ga^{+3}. For example, in relation to multiply phosphorylated peptides, Zhou states that "the binding [to Fe^{+3}] is sufficiently strong that the multiphosphorylated peptides are not easily dissociated from the agarose by laser."[3] In contrast, binding to Ga^{+3} allows release of multiply phosphorylated peptides without phosphatase treatment.[4] In addition, in relation to singly phosphorylated peptides, Zhou states that "Ga^{3+} has weaker affinity for the monophosphorylated peptides than does Fe^{3+}-loaded agarose."[5]

Zhou thus is teaching away from replacing iron with gallium in the binding assays of Nikiforov, because Zhou is suggesting that gallium has a lower affinity for phosphopeptides than iron. Accordingly, one of skill in the art would be motivated not to replace iron with gallium in the binding assays of Nikiforov, because one of skill in the art would have expected gallium to perform worse than iron in those assays.

Thus, for at least these reasons, applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 103(a) and prompt allowance of the pending claims.

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[3] Zhou, pg. 278, second column, first paragraph.
[4] Id., Figure 1 and pg. 276, first column, first paragraph.
[5] Id., pg. 280, first column, first paragraph.

B. The Claimed Invention has Unexpected Benefits

Applicants have found at least three significant unexpected benefits[6] to using gallium in luminescence polarization assays that are neither taught nor suggested by Nikiforov, Zhou, or any other prior art reference of record. The unexpected benefits are:

- First, gallium, in contrast to iron, enhances intensity, instead of quenching intensity, after associating with luminescent assay components. Consequently, assays employing gallium can be performed much more quickly and with much less statistical noise than assays employing iron.

- Second, assays employing gallium, in contrast to assays employing iron, have a much greater dynamic range of polarization. Consequently, assays employing gallium are much more robust and easy to perform than assays employing iron, if the latter can be performed at all.

- Third, assays employing gallium to bind product can better distinguish the existence of product in a mixture of substrate and product, relative to iron, since product bound to gallium will contribute more rather than less to the total polarization.

The first advantage, at least, was presented in the application, as filed.[7] Moreover, all three advantages augment the teach-away argument set forth above.

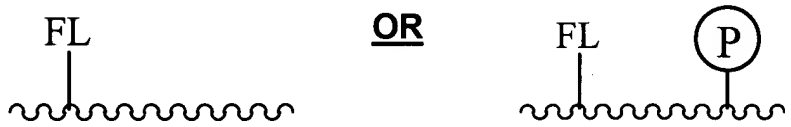
Experimental results detailing these unexpected benefits are described in a Declaration from Dr. Richard Sportsman, an expert on luminescence polarization assays, and one of the inventors on the subject application. The Declaration is included as Exhibit A, and the results are summarized below.

1. Overview of Experimental Procedure

Applicants performed a series of binding assays to compare reagents that include iron ("iron reagent") or gallium ("gallium reagent"). Each binding assay included

[6] See MPEP §716.02.
[7] See, e.g., Application, page 50, lines 12 and 13.

either a fluorescent substrate (a luminophore-labeled, nonphosphorylated peptide; "S") or a fluorescent product (a phosphorylated form of the peptide; "P"), as follows:



"Substrate" "Product"

The product corresponds to a product that would be produced by operation of a suitable kinase enzyme on the substrate. The fluorescent substrate or product was incubated with the iron reagent or the gallium reagent. Then, the effect of each metal on luminescence intensity and polarization from the substrate or product was measured. Accordingly, these binding assays with gallium correspond to an embodiment of the invention for detecting kinase enzyme activity in which there is no enzyme activity (substrate (S) only) or substantial enzyme activity (product (P) only). Alternatively, by reversing the substrate and product designations of these peptides, the results correspond to an embodiment of the invention for detecting phosphatase enzyme activity in which there is substantial enzyme activity or no enzyme activity, with similar conclusions. Results corresponding to embodiments of the invention in which there are intermediate enzyme activities also are described below.

2. *Advantage 1: Unexpected Benefits on Intensity Measurements*

The experimental results presented in the Declaration show that gallium provides at least one significant unexpected benefit over iron for measurements of luminescence

intensity. Importantly, measurements of luminescence intensity underlie all measurements of luminescence polarization.[8]

Figure 1 of the Declaration, which is reproduced below, shows results of total luminescence intensity measurements in relative fluorescence units (RFU), as a function of added metal (iron reagent or gallium reagent) and peptide phosphorylation state (P or S) in the binding assays:

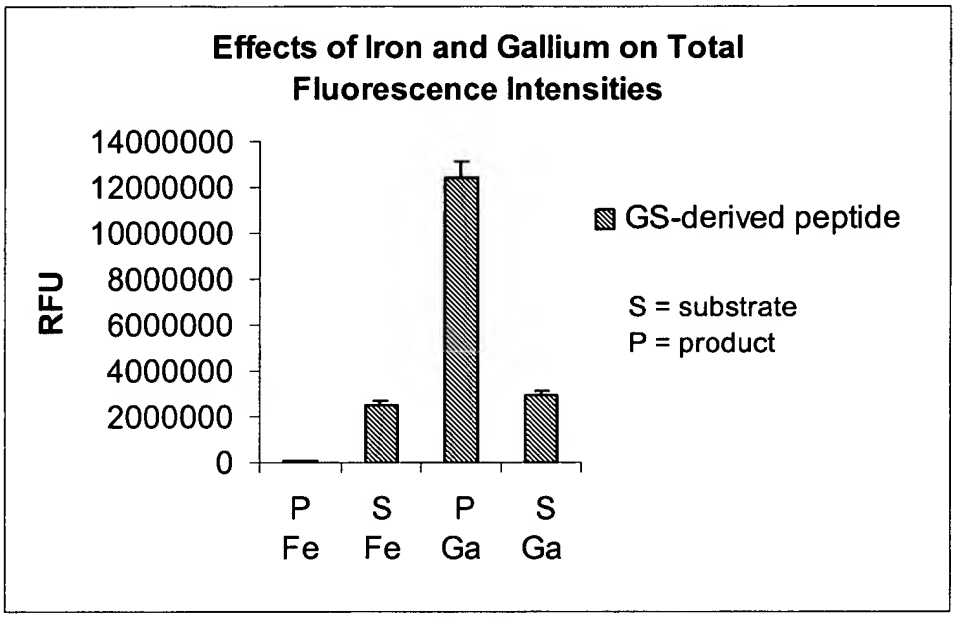


Figure 1

The luminescence intensity (or brightness) generally was comparable in assays of the substrate (S) performed with either the iron reagent or the gallium reagent. These results are consistent with little or no binding of each reagent to this substrate, and thus

[8] The relationship between polarization and intensity is expressed by the following equation:

$$P = \frac{I_{||} - I_{\perp}}{I_{||} + I_{\perp}}$$

Here, P is the polarization, $I_{||}$ is the intensity of luminescence polarized parallel to the polarization of the excitation light, and $I_{\perp}$ is the intensity of luminescence polarized perpendicular to the polarization of the excitation light, all following excitation with polarized light. (This equation can be modified further to include a "G factor" that corrects the intensities for instrumental errors; see Declaration, paragraph 11.) Thus, measurements of polarization are only as good as the underlying measurements of intensity. See, e.g., Application, page 59, line 20, to page 60 line 4.

little or no effect on the intensity of luminophore associated with this substrate. In contrast, the luminescence intensity (or brightness) differed dramatically according to which of these reagents was present in assays of the product (P). In particular, the intensity was about one-hundred-fold higher in assays of the product performed with the gallium reagent relative to assays of the product performed with the iron reagent. This dramatic difference in intensity was produced, in part, by an approximately four-fold increase in the intensity of the product relative to substrate (S) for the gallium reagent, and, in part, by a more than twenty-fold decrease in the intensity of the product relative to substrate for the iron reagent. Therefore, in contrast to the iron reagent, which quenched luminescence intensity substantially when present with product and its associated luminophore, the gallium reagent not only did not quench but actually enhanced this intensity.

These dramatic differences in intensity between the iron reagent and the gallium reagent translate into dramatic differences in the timing and sensitivity of assays performed with these reagents. Polarization assays on large numbers of samples are configured to be analyzed as fast as possible, to maximize throughput. For example, the SmartRead™ system employed in polarization readers produced by Molecular Devices Corporation is designed to collect data on each sample until a threshold number of photons (or a timeout period) has been reached, and then move on to the next sample. This threshold number of photons typically is selected to correspond to a minimally acceptable signal-to-noise ratio for data analysis.[9] Thus, due to the opposing effects of iron and gallium on brightness, it would take about 100 times as long to collect

[9] The signal-to-noise ratio of the intensity in photon processes is proportional to the square root of the number of photons collected.

comparable light per sample with the iron reagent as with the gallium reagent. This difference literally is the difference between practical and impractical in high-throughput drug screening, since assay measurements that take 20-40 milliseconds to perform with gallium would take a completely unacceptable 2-4 seconds to perform with iron! Moreover, in any context, this difference means that for a given measurement time the signal strength (and thus the signal-to-noise ratio) will be significantly higher in gallium-based polarization assays than in iron-based polarization assays.

The dramatic intensity advantages of gallium relative to iron are not obvious. To the contrary, metals are well-known luminescence quenchers (i.e., extinguishers). Indeed, Pierce Biotechnology sells a kinase assay system in which enzyme activity is observed using luminescence quenching that accompanies interaction of a fluorescently labeled phosphorylated peptide with iron; see Exhibit B.[10] Specifically, in the Pierce assay, luminescence intensity decreases monotonically with increasing phosphorylation, increasing kinase concentration, and increasing time, all reflecting increasing association of iron with the luminophore.[11] In contrast, applicants have discovered that gallium, unlike iron, not only does not quench but instead actually enhances intensity when bound to a luminophore. None of the references of record, including Nikiforov and Zhou, teach or suggest this unexpected and patentable benefit of using a binding partner including gallium in polarization assays.

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[10] See, e.g., Pierce IQ® Assay Platform: Technical Handbook (Pierce Pub. No. 1600963) (August 2003). This handbook is included with this Response as Exhibit B.

[11] Id.

3. *Advantages 2 and 3: Unexpected Benefits on Polarization Measurements*

The experimental results presented in the Declaration also show that gallium provides at least two significant unexpected benefits over iron in distinguishing product from substrate in polarization assays.

First, gallium-based polarization assays have significantly higher dynamic ranges than iron-based polarization assays, enhancing the speed at which assays can be performed. (This enhancement in speed comes in addition to the enhancement described above relating to differences in intensity.) Figure 2 of the Declaration, which is reproduced below, shows a plot of luminescence polarization (mP) measured as a function of added metal (iron or gallium) and peptide phosphorylation state:

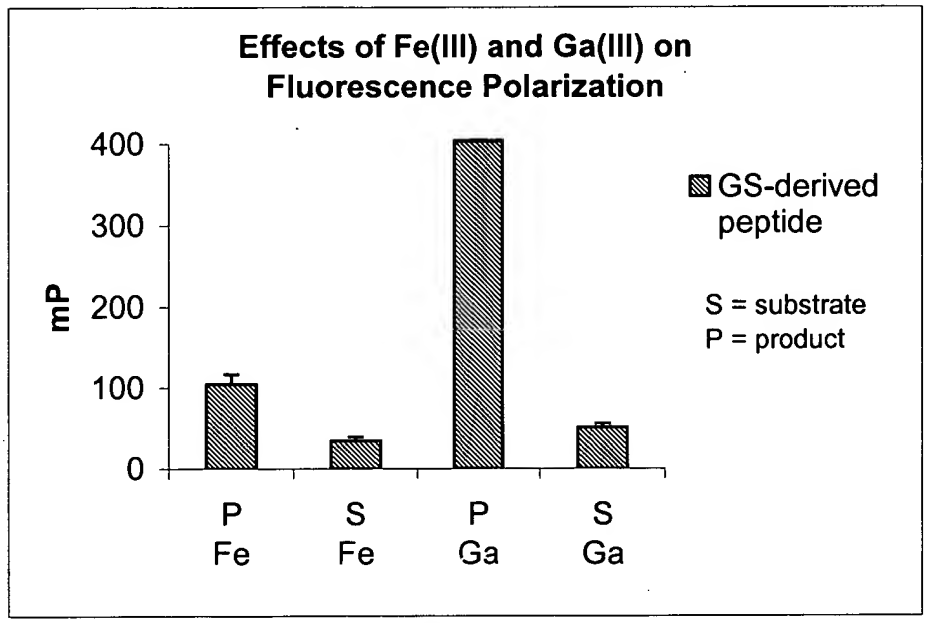

Figure 2

The "dynamic range" is determined by the difference between the degree of polarization produced by luminophore-associated product and substrate. In the configurations tested, the iron reagent provides a dynamic range of about 70 mP, and the gallium

reagent provides a dynamic range of about 350 mP.[12] Thus, because it is much easier to measure a change of 350 mP than a change of 70 mP (since the larger change will be more significant relative to noise than the smaller change), it can take significantly less time to collect enough light to get good enough statistics reliably to measure the large change in polarization in gallium-based assays than the small change in polarization in iron-based assays.

Second, gallium-based polarization assays may be significantly more capable of detecting binding in assays involving mixtures of substrate and product (e.g., assays in which there is only intermediate enzyme activity) than iron-based polarization assays. Such "intermediate enzyme activity" can be a common experimental situation, reflecting intermediate activity of the enzyme itself, intermediate activity of putative modulators of the enzyme (e.g., in a screening assay), and/or intermediate progress in reactions mediated by the enzyme (e.g., brought about by prematurely stopping the assay). The net polarization of a mixture of product and substrate depends on the separate polarizations of their associated luminophores, weighted in part by their relative intensities. Therefore, quenched luminophores, produced with the iron reagent, will contribute to the net polarization only minimally, if at all, because their luminescence emissions are quenched. Thus, the net polarization of a mixture in which bound luminophore is quenched by iron will arise mostly or exclusively from unbound luminophore, making corresponding binding assays insensitive to the binding that they are supposed to detect. In contrast, the net polarization of a mixture in which the

[12] The minimum possible dynamic range is 0 mP, and the maximum possible dynamic range is 500 mP, for randomly oriented molecules in a polarization assay. See, e.g. Application, page 60, lines 11 and 12. Thus, the dynamic range for Fe(III)-based polarization assays lies near the bottom of the possible range, and the dynamic range for Ga(III)-based polarization assays lies near the top of the possible range.

luminescence of bound luminophore is enhanced by gallium will arise preferentially from bound luminophore, allowing more sensitive (rather than less sensitive) detection of binding in a mixture of substrate and product.

None of the references of record, including Nikiforov and Zhou, teach or suggest these additional unexpected and patentable benefits of using a binding partner including gallium in polarization assays.

4. Summary of Experimental Results

The comparison of the iron reagent and the gallium reagent, presented above, indicates that the gallium reagent is dramatically superior to the iron reagent in at least three aspects. First, the gallium reagent, relative to the iron reagent, permits detectable emission of about one-hundred fold more light from a bound, exemplary luminophore. Second, the gallium reagent has a large, near maximal dynamic range of polarization, while the iron reagent has a small, near minimal dynamic range of polarization. Third, the gallium reagent can better distinguish the existence of product in a mixture of substrate and product, relative to the iron reagent, since product bound to the gallium reagent will contribute preferentially more rather than preferentially less to the total polarization. Therefore, with the configurations tested, the use of the gallium reagent in place of the iron reagent converts a binding assay that effectively is inoperable into one that is very robust. None of the references of record, including Nikiforov and Zhou, teach or suggest any of these unexpected and patentable benefits of using a binding partner including gallium in polarization assays.

The shortcomings of iron presented here are consistent with applicants' observation that there do not appear to be any non-gallium metal-based polarization assays. The Nikiforov patent does not disclose data in which a metal was used in a

binding partner, nor to applicants' knowledge has Nikiforov published any papers showing such an assay. Furthermore, Caliper Life Sciences, the owner of the Nikiforov patent, does not appear to have introduced a product based on polarization assays using iron, or any other metal, judging from applicants' March 27, 2006, review of Caliper's website. In contrast, applicants have developed a variety of commercial products that are now for sale for detecting the activity of over twenty different enzymes using gallium-based polarization assays.

In summary, the unexpected benefits and superior properties demonstrated by the claimed assay rebut any assertion of *prima facie* obviousness by the Examiner. Thus, for at least these additional reasons, applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 103(a) and prompt allowance of the pending claims.

II. Conclusion

Applicants believe that this communication is fully responsive to the Office Action, and that the claims are currently in condition for allowance. However, if there are any remaining matters, or if it would otherwise advance prosecution of the application, the Examiner is encouraged to call the undersigned attorney at (503) 224-6655.

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop AMENDMENT, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450 on April 4, 2006.

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